Journal of Biochemistry

Vol.167, No.3（2020 年 3 月 発 行）

ダイジェスト

JB SPECIAL ISSUE—DYNAMICS REGULATION OF MITOCHONDRIA
GUEST EDITORS: NAOTADA ISHIHARA AND HOWARD JACOBS

JB Special Issue—Reviews

PINK1 import regulation at a crossroad of mitochondrial fate: the molecular mechanisms of PINK1 import

Shiori Sekine[1]
[1]Aging Institute, Division of Cardiology, Department of Medicine, University of Pittsburgh, Pittsburgh, PA 15219, USA

PTEN-induced kinase 1 (PINK1) is a mitochondrial kinase whose activity is tightly regulated by the mitochondrial health status. In response to mitochondrial damage, activated PINK1 can promote mitophagy, an autophagic elimination of damaged mitochondria, by cooperating with Parkin ubiquitin ligase. Loss-of-function of PINK1/Parkin-mediated mitophagy results in the accumulation of dysfunctional mitochondria, which could be one aetiology of Parkinson's disease (PD). Within step-by-step signalling cascades of PINK1/Parkin-mediated mitophagy, mitochondrial damage-dependent PINK1 kinase activation is a critical step to trigger the mitophagy signal. Recent investigation of this process reveals that this stress-dependent PINK1 kinase activation is achieved by its regulated import into different mitochondrial compartments. Thus, PINK1 import regulation stands at an important crossroad to determine the mitochondrial fate—'keep' or 'remove'? In this review, we will summarize how the PINK1 import is regulated in a mitochondrial health status-dependent manner and how this process could be pharmacologically modulated to activate the PINK1/Parkin pathway.

Keywods: mitochondrial import, mitochondrial protease, mitophagy, Parkinson's disease, PINK1

Mass spectrometry-based methods for analysing the mitochondrial interactome in mammalian cells

Takumi Koshiba[1] and Hidetaka Kosako[2]
[1]Department of Chemistry, Faculty of Science, Fukuoka University, Fukuoka 814–0180, Japan, [2]Division of Cell Signaling, Fujii Memorial Institute of Medical Sciences, Tokushima University, Tokushima 770–8503, Japan

Protein–protein interactions are essential biologic processes that occur at inter- and intracellular levels. To gain insight into the various complex cellular functions of these interactions, it is necessary to assess them under physiologic conditions. Recent advances in various proteomic technologies allow to investigate protein–protein interaction networks in living cells. The combination of proximity-dependent labelling and chemical cross-linking will greatly enhance our understanding of multi-protein complexes that are difficult to prepare, such as organelle-bound membrane proteins. In this review, we describe our current understanding of mass spectrometry-based proteomics mapping methods for elucidating organelle-bound membrane protein complexes in living cells, with a focus on protein–protein interactions in mitochondrial subcellular compartments.

Keywods: BioID, mass spectrometry, mitochondria, proteome, XL-MS

Mitochondrial division, fusion and degradation

Daisuke Murata[1], Kenta Arai[1], Miho Iijima[1] and Hiromi Sesaki[1]
[1]Department of Cell Biology, Johns Hopkins University School of Medicine, 725 N. Wolfe St., Baltimore, MD 21205, USA

The mitochondrion is an essential organelle for a wide range of cellular processes, including energy production, metabolism, signal transduction and cell death. To execute these functions, mitochondria regulate their size, number, morphology and distribution in cells via mitochondrial division and fusion. In addition, mitochondrial division and fusion control the autophagic degradation of dysfunctional mitochondria to maintain a healthy population. Defects in these dynamic membrane processes are linked to many human diseases that include metabolic syndrome, myopathy and neurodegenerative disorders. In the last several years, our fundamental understanding of mitochondrial fusion, division and degradation has been significantly advanced by high resolution structural analyses, protein-lipid biochemistry, super resolution microscopy and *in vivo* analyses using animal models. Here, we summarize and discuss this exciting recent progress in the mechanism and function of mitochondrial division and fusi on.

Keywods: actin, dynamin-related GTPase, ER-mitochondria contact, lipids, mitophagy

The 'mitochondrial contact site and cristae organising system' (MICOS) in health and human disease

Matthew J Eramo[1], Valerie Lisnyak[1], Luke E Formosa[1] and Michael T Ryan[1]
[1]Department of Biochemistry and Molecular Biology, Monash Biomedicine Discovery Institute, 23 Innovation Walk, Monash University, 3800 Melbourne, Victoria, Australia

The 'mitochondrial contact site and cristae organising system' (MICOS) is an essential protein complex that promotes the formation, maintenance and stability of mitochondrial cristae. As such, loss of core MICOS components disrupts cristae structure

and impairs mitochondrial function. Aberrant mitochondrial cristae morphology and diminished mitochondrial function is a pathological hallmark observed across many human diseases such as neurodegenerative conditions, obesity and diabetes mellitus, cardiomyopathy, and in muscular dystrophies and myopathies. While mitochondrial abnormalities are often an associated secondary effect to the pathological disease process, a direct role for the MICOS in health and human disease is emerging. This review describes the role of MICOS in the maintenance of mitochondrial architecture and summarizes both the direct and associated roles of the MICOS in human disease.

Keywods: cristae, membrane organization, MICOS, mitochondria

Mitochondrial dynamics and interorganellar communication in the development and dysmorphism of mammalian oocytes

Osamu Udagawa[1] and Naotada Ishihara[2,3]

[1]Center for Health and Environmental Risk Research, Integrated Health Risk Research Section, National Institute for Environmental Studies, 16–2 Onogawa, Tsukuba, Ibaraki 305–8506, Japan, [2]Department of Biological Science, Graduate School of Science, Osaka University, 1–1 Machikaneyama, Toyonaka, Osaka 560–0043, Japan, [3]Department of Protein Biochemistry, Institute of Life Science, Kurume University, 67 Asahi, Kurume, Fukuoka 830–0011, Japan

Mitochondria play many critical roles in cells, not only by supplying energy, but also by supplying metabolites, buffering Ca^{2+} levels and regulating apoptosis. During oocyte maturation and subsequent embryo development, mitochondria change their morphology by membrane fusion and fission, and coordinately undergo multiple cellular events with the endoplasmic reticulum (ER) closely apposed. Mitochondrial fusion and fission, known as mitochondrial dynamics, are regulated by family members of dynamin GTPases. Oocytes in animal models with these regulators artificially altered exhibit morphological abnormalities in nearby mitochondria and at the ER interface that are reminiscent of major cytoplasmic dysmorphisms in human assisted reproductive technology, in which a portion of mature oocytes retrieved from patients contain cytoplasmic dysmorphisms associated with mitochondria and ER abnormal morphologies. Understanding organelle morpho-homeostasis in oocytes obtained from animal models will contribute to the development of novel methods for determining oocyte health and for how to deal with dysmorphic oocytes.

Keywods: cytoplasmic dysmorphisms, dynamin-related GTPase, mitochondrial fission and fusion, oocyte maturation, organelle morphology

Regulation of growth in Drosophila melanogaster: the roles of mitochondrial metabolism

Howard T Jacobs[1], Jack George[1] and Esko Kemppainen[1]

[1]Faculty of Medicine and Health Technology, FI-33014 Tampere University, Finland

Mitochondrial functions are often considered purely from the standpoint of catabolism, but in growing cells they are mainly dedicated to anabolic processes, and can have a profound impact on the rate of growth. The ***Drosophila*** larva, which increases in body mass ～200-fold over the course of ～3days at 25°C, provides an excellent model to study the underlying regulatory machinery that connects mitochondrial metabolic capacity to growth. In this review, we will focus on several key aspects of this machinery: nutrient sensing, endocrine control of feeding and nutrient mobilization, metabolic signalling, protein synthesis regulation and pathways of steroid biosynthesis and activity. In all these aspects, mitochondria appear to play a crucial role.

Keywods: ecdysone, insulin signalling, PGC-1, proteostasis, pyruvate

JB Special Issue—Regular Papers

A AAA ATPase Cdc48 with a cofactor Ubx2 facilitates ubiquitylation of a mitochondrial fusion-promoting factor Fzo1 for proteasomal degradation

Sabiqun Nahar[1,2], Abhijit Chowdhury[1,2], Teru Ogura[1,2] and Masatoshi Esaki[1]

[1]Department of Molecular Cell Biology, Institute of Molecular Embryology and Genetics, Kumamoto University, Kumamoto 860–0811, Japan, [2]Program for Leading Graduate Schools "HIGO Program", Kumamoto University, Kumamoto 860–8556, Japan

Dynamic functionality of mitochondria is maintained by continual fusion and fission events. A mitochondrial outer membrane protein Fzo1 plays a pivotal role upon mitochondrial fusion by homo-oligomerization to tether fusing mitochondria. Fzo1 is tightly regulated by ubiquitylations and the ubiquitin-responsible AAA protein Cdc48. Here, we show that a Cdc48 cofactor Ubx2 facilitates Fzo1 turnover. The Cdc48-Ubx2 complex has been shown to facilitate degradation of ubiquitylated proteins stacked at the protein translocation complex in the mitochondrial outer membrane by releasing them from the translocase. By contrast, in the degradation process of Fzo1, the Cdc48-Ubx2 complex appears to facilitate the degradation-signalling ubiquitylation of the substrate itself. In addition, the Cdc48-Ubx2 complex interacts with Ubp2, a deubiquitylase reversing the degradation-signalling ubiquitylation of Fzo1. These results suggest that the Cdc48-Ubx2 complex regulates Fzo1 turnover by modulating ubiquitylation status of the substrate.

Keywods: AAA ATPase, Cdc48, degradation, mitochondria, ubiquitin

Mitochondrial nucleoid morphology and respiratory function are altered in Drp1-defi cient HeLa cells

Azusa Ota[1,2], Takaya Ishihara[1,2] and Naotada Ishihara[1,2]

[1]Department of Biological Sciences, Graduate School of Science, Osaka University, 1–1 Machikaneyama-machi, Toyonaka, Osaka 560–0043, Japan, [2]Department of Protein Biochemistry, Institute of Life Science, Kurume University, 67 Asahi-machi, Kurume, Fukuoka 830–0011, Japan

Mitochondria are dynamic organelles that frequently divide and fuse with each other. The dynamin-related GTPase protein Drp1 has a key role in mitochondrial fission. To analyse the physiological roles of Drp1 in cultured human cells, we analysed Drp1-deficient HeLa cells established by genome editing using CRISPR/Cas9. Under fluorescent microscopy, not only mitochondria were elongated but their DNA (mtDNA) nucleoids were extremely enlarged in bulb-like mitochondrial structures ('mito-bulbs') in the Drp1-deficient HeLa cells. We further found that respiratory activity, as measured by oxygen consumption rates, was severely repressed in Drp1-deficient HeLa cells and that this was reversible by the co-repression of mitochondrial fusion factors. Although mtDNA copy number was not affected, several respiratory subunits were repressed in Drp1-deficient HeLa cells. These results suggest that mitochondrial fission is required for the maintenance of active respiratory activity and the morphology of mtDNA nucleoids in human cells.

Keywods: GTPase, membrane dynamics, mitochondria, mtDNA, respiratory complex

BIOCHEMISTRY

Biochemistry General

LncRNA MEG3 inhibits the progression of prostate cancer by facilitating H3K27 trimethylation of EN2 through binding to EZH2

Yaojun Zhou[1], Hongqiong Yang[2], Wei Xia[1], Li Cui[1], Renfang Xu[1], Hao Lu[1], Dong Xue[1], Zinong Tian[1], Tao Ding[1], Yunjie Cao[1], Qianqian Shi[1] and Xiaozhou He[1]

[1]Department of Surgical Urology, The Third Affiliated Hospital of Soochow University, No. 185 Juqian Street, Changzhou 213003, Jiangsu Province, China, [2]Department of Geriatric Medicine, The Third Affiliated Hospital of Soochow University, No. 185 Juqian Street, Changzhou 213003, Jiangsu Province, China

This study aims to study the effects of intra-nuclear lncRNA MEG3 on the progression of prostate cancer and the underlying mechanisms. Expressions of relative molecules were detected by Quantitative real time PCR (qRT-PCR) and western blot. Chromatin immunoprecipitation and RNA immunoprecipitation (RIP) assays were used to evaluate the interaction between intra-nuclear MEG3, histone methyltransferase EZH2 and Engrailed-2 (EN2). The impacts of MEG3 on the viability, proliferation and invasion of prostate cancer cells (PC3) were evaluated by methyl thiazolyl tetrazolium, colony formation and transwell assays, respectively. PC3 cells were transfected with MEG3 and transplanted into nude mice to analyse the effect of MEG3 on tumourigenesis of PC3 cells *in vivo*. EN2 expression was inversely proportional to MEG3 in the prostate cancer tissues and PC3 cells. RIP results showed that intra-nuclear MEG3 could bind to EZH2. Knockdown of MEG3 and/or EZH2 up-regulated EN2 expression and reduced the recruitment of EZH2 and H3K-27me3 to EN2, while over-expressed MEG3 caused opposite effects. MEG3 over-expression suppressed cell viability, colony formation, cell invasion and migration of PC3 cells *in vitro* and inhibited tumourigenesis of PC3 cells *in vivo*, while EN2 over-expression diminished the effects. These findings indicated that MEG3 facilitated H3K27 trimethylation of EN2 via binding to EZH2, thus suppressed the development of prostate cancer.

Keywods: EH2, EZH2, histone methylation, lncRNA MEG3, prostate cancer

Enzymology

Role of the N-terminus in human 4-hydroxyphenylpyruvate dioxygenase activity

An-Ning Feng[1], Chih-Wei Huang[2,3], Chi-Huei Lin[4], Yung-Lung Chang[4], Meng-Yuan Ni[4] and Hwei-Jen Lee[4]

[1]Department of Cardiology, Cheng Hsin General Hospital, No. 45, Cheng Hsin St. Pai-Tou, Taipei 11220, Taiwan, [2]Department of Pharmacy Practice, Tri-Service General Hospital, No. 325, Sec. 2, Chenggong Rd., Neihu, Taipei 11490, Taiwan, [3]School of Pharmacy, National Defense Medical Center, No. 161, Sec. 6, Minchuan East Rd., Neihu, Taipei 11490, Taiwan, [4]Department of Biochemistry, National Defense Medical Center, No. 161, Sec. 6, Minchuan East Rd., Neihu, Taipei 11490, Taiwan

4-Hydroxyphenylpyruvate dioxygenase (HPPD) is a key enzyme in tyrosine catabolism, catalysing the oxidation of 4-hydroxyphenylpyruvate to homogentisate. Genetic deficiency of this enzyme causes type III tyrosinaemia. The enzyme comprises two barrel-shaped domains formed by the N- and C-termini, with the active site located in the C-terminus. This study investigated the role of the N-terminus, located at the domain interface, in HPPD activity. We observed that the k_{cat}/K_m decreased ～8-fold compared with wild type upon removal of the 12 N-terminal residues (ΔR13). Interestingly, the wild-type level of activity was retained in a mutant missing the 17 N-terminal residues, with k_{cat}/K_m 11-fold higher than that of the ΔR13 mutant; however, the structur al stability of this mutant was lower than that of wild type. A 2-fold decrease in catalytic efficiency was observed for the K10A and E12A mutants, indicating synergism between these residues in the enzyme catalytic function. A molecular dynamics simulati on showed large RMS fluctuations in ΔR13 suggesting that conf ormational flexibility at the domain interface leads to lower activ ity in this mutant. These results demonstrate that the N-terminus

maintains the stability of the domain interface to allow for cataly sis at the active site of HPPD.

Keywods: 4-hydroxyphenylpyruvate dioxygenase, molecular dynamics simulation, N-terminal segment, truncated mutation, tyrosine catabolism

Gene cloning and characterization of thiourocanate hydratase from Burkholderia sp. HME13

Hisashi Muramatsu[1], Haruna Miyaoku[2], Syuya Kurita[2], Hidenori Matsuo[3], Takehiro Kashiwagi[1], Chul-Sa Kim[1], Motoko Hayashi[4], Hiroaki Yamamoto[4], Shin-Ichiro Kato[1] and Shinji Nagata[1]

[1]Multidisciplinary Science Cluster, Research and Education Faculty, Kochi University, B200 Monobe, Nankoku, Kochi 783–8502, Japan, [2]Department of Agriculture, Kochi University, B200 Monobe, Nankoku, Kochi 783–8502, Japan, [3]Graduate School of Integrated Arts and Sciences, Kochi University, B200 Monobe, Nankoku, Kochi 783–8502, Japan, [4]Corporate Research Center, R&D Headquaters Management, Daicel Corporation 1–1 Shinko-cho, Myoko, shi, Niigata 944–8550, Japan

A novel enzyme, thiourocanate hydratase, which catalyses the conversion of thiourocanic acid to 3-(5-oxo-2-thioxoimidazolidin-4-yl) propionic acid, was isolated from the ergothioneine-utilizing strain, *Burkholderia* sp. HME13. When the HME13 cel ls were cultured in medium containing ergothioneine as the sole nitrogen source, thiourocanate-metabolizing activity was detected in the crude extract from the cells. However, activity was not detected in the crude extract from HME13 cells that were cultured in Luria-Bertani medium. The gene encoding thiourocanate hydratase was cloned and expressed in *Escherichia coli*, and the recombinant enzyme was purified to homogeneity. The enzyme showed maximum activity at pH 7.5 and 55°C and was stable between pH 5.0 and 10.5, and at temperatures up to 45°C. The K_m and V_{max} values of thiourocanate hydratase towards thiourocanic acid were 30 μM and 7.1 μmol/min/mg, respectively. The enzyme was strongly inhibited by $CuCl_2$ and $HgCl_2$. The amino acid sequence of the enzyme showed 46% identity to urocanase from Pseudomonas putida, but thiourocanate hydratase had no urocanase activity.

Keywods: ergothioneine, thiourocanic acid, thiourocanate hydratase, urocanase, Burkholdria, Abbreviations: 2-ME, 2-mercaptoethanol, IPTG, isopropyl-β-D-thiogalactopyranoside, LB, Luria-Bertani, PMSF, phenylmethylsulfonyl fluoride, UPLC, ultra-performance liquid chromatography

Metabolism and Bioenergetics

Interleukin-6 confers radio-resistance by inducing Akt-mediated glycolysis and reducing mitochondrial damage in cells

Neeraj Kumari[1,2], Asmita Das[2] and Anant Narayan Bhatt[1]

[1]Division of Radiation Biosciences, Institute of Nuclear Medicine and Allied Sciences, Brig. S K Mazumdar Marg, Delhi 110054, India, [2]Department of Biotechnology, Delhi Technological University, Main Bawana Road, Delhi 110042, India

Interleukin-6 (IL-6)-induced glycolysis and therapeutic resistance is reported in some cell systems; however, the mechanism of IL-6-induced glycolysis in radio-resistance is unexplored. Therefore, to investigate, we treated Raw264.7 cells with IL-6 (1 h prior to irradiation) and examined the glycolytic flux. Increased expression of mRNA and protein levels of key glycolytic enzymes was observed after IL-6 treatment, which conferred glycolysis dependent resistance from radiation-induced cell death. We further established that IL-6-induced glycolysis is activated by Akt signalling and knocking down Akt or inhibition of pan Akt phosphorylation significantly abrogated the IL-6-induced radio-resistance. Moreover, reduction of IL-6-induced pAkt level suppressed the expression of Hexokinase-2 and its translocation to the mitochondria, thereby inhibiting the glycolysis-induced resistance to radiation. IL-6-induced glycolysis also minimized the radiation-induced mitochondrial damage. These results suggest that IL-6-induced glycolysis observed in cells may be responsible for IL-6-mediated therapeutic radio-resistance in cancer cells, partly by activation of Akt signalling.

Keywods: Akt signalling, glycolysis, hexokinase-2, IL-6, radio-resistance

Biochemistry in Diseases and Aging

Effect of redox imbalance on protein modifi cations in lymphocytes of psoriatic patients

Piotr Wójcik[1], Agnieszka Gęgotek[1], Adam Wroński[2], Anna Jastrząb[1], Agnieszka Żebrowska[3] and Elżbieta Skrzydlewska[1]

[1]Department of Analytical Chemistry, Medical University of Bialystok, Mickiewicza 2D, 15–222 Białystok, Poland, [2]Dermatological Specialized Center "DERMAL" NZOZ in Białystok, Nowy Świat 17/5, 15–453 Białystok, Poland, [3]Regional Center for Blood Donation and Blood Treatment, M. Skłodowskiej-Curie 23, 15–950 Białystok, Poland

Lymphocytes are one of the most important cells involved in the pathophysiology of psoriasis; therefore, the aim of this study was to assess the redox imbalance and protein modifications in the lymphocytes of patients with psoriasis vulgaris (PsV) or psoriatic arthritis (PsA). The results show a stronger shift in redox status to pro-oxidative conditions (observed as an increased reactive oxygen species level, a decrease in catalase activity and lower levels of glutathione peroxidase and vitamin E compared with healthy controls) in the lymphocytes of PsA than PsV patients. It is also favoured by the enhanced level of activators of the Nrf2 transcription factor in lymphocytes of PsV compared with decreased of these proteins level in PsA. Moreover, the differential modifications of proteins by lipid peroxidation products 4-oxononenal (mainly binding proteins) and malondialdehyde (mainly catalytic proteins with redox activity), promoted a pro-

apoptotic pathway in lymphocytes of PsV, which was manifested by enhanced expression of pro-apoptotic caspases, particularly caspase 3. Taken together, differences in Nrf2 pathway activation may be responsible for the differential level of redox imbalance in lymphocytes of patients with PsV and PsA. This finding may enable identification of a targeted therapy to modify the metabolic pathways disturbed in psoriasis.

Keywods: apoptosis, lymphocytes, protein modifications, psoriasis, redox balance

Journal of Biochemistry

Vol.167, No.4（2020 年 4 月 発 行）

ダイジェスト

JB Review Featured article of the month

Tactics of cancer invasion: solitary and collective invasion

Tomoaki Nagai[1], Tomohiro Ishikawa[2], Yasuhiro Minami[3] and Michiru Nishita[1]

[1]Department of Biochemistry, Fukushima Medical University School of Medicine, 1 Hikariga-oka, Fukushima 960–1295, Japan, [2]Department of Life and Environmental System Science, Graduate School of Nanobioscience, Yokohama City University, 22–2 Seto, Kanazawa-ku, Yokohama 236–0027, Japan, [3]Division of Cell Physiology, Department of Physiology and Cell Biology, Kobe University Graduate School of Medicine, Kobe 650–0017, Japan

Much attention has been paid on the mechanism of cancer invasion from the viewpoint of the behaviour of individual cancer cells. On the other hand, histopathological analyses of specimens from cancer patients and of cancer invasion model animals have revealed that cancer cells often exhibit collective invasion, characterized by sustained cell-to-cell adhesion and polarized invasion as cell clusters. Interestingly, it has recently become evident that during collective invasion of cancer cells, the cells localized at invasion front (leader cells) and the cells following them (follower cells) exhibit distinct cellular characteristics, and that there exist the cells expressing representative proteins related to both epithelial and mesenchymal properties simultaneously, designated as hybrid epithelial-to-mesenchymal transition (EMT)-induced cells, in cancer tissue. Furthermore, the findings that cells adopted in hybrid EMT state form clusters and show collective invasion *in vitro* emphasize an importance of hybrid EMT-induced cells in collective cancer invasion. In this article, we overview recent findings of the mechanism underlying collective invasion of cancer cells and discuss the possibility of controlling cancer invasion and metastasis by targeting this process.

Keywords: cell migration, cell polarity, epithelial-to-mesenchymal transition (EMT), follower cells, leader cells

BIOCHEMISTRY

Biochemistry General

TXNIP induced by MondoA, rather than ChREBP, suppresses cervical cancer cell proliferation, migration and invasion

Junhua Zhang[1], Xingbo Tian[2], Huifang Yin[2], Songshu Xiao[2], Shuijing Yi[2], Youzhong Zhang[1] and Fei Zeng[2]

[1]Department of Gynecology and Obstetrics, Qilu Hospital of Shandong University, No. 107 West Wenhua Road, Jinan 250012, Shandong Province, China, [2]Department of Gynecology and Obstetrics, The Third Xiangya Hospital of Central South University, No. 138 Tongzipo Road, Changsha 410013, Hunan Province, China

Evidence has indicated the associations between thioredoxin-interacting protein (TXNIP) and cancers. However, the role of TXNIP in cervical cancer remains unclear. Hence, this study aims to investigate the role of TXNIP in regulating cervical cancer cell proliferation, migration and invasion. TXNIP expression can be regulated by either MondoA or ChREBP in a cell- or tissue- dependent manner. Thus, we also explored whether TXNIP expression in cervical cancer can be regulated by MondoA or ChREBP. Our results showed that TXNIP expression was decreased in cervical cancer cells (HeLa, SiHa, CaSki, MS751, C-33A). Furthermore, TXNIP overexpression inhibited cell proliferation, migration and invasion in HeLa cells, whereas TXNIP silencing exerted the opposite effect in C-33A cells. Moreover, TXNIP expression could be induced by MondoA, rather than ChREBP in HeLa cells. Additionally, MondoA overexpression inhibited cell proliferation, migration and invasion through upregulating TXNIP in HeLa cells. In summary, TXNIP induced by MondoA, rather than ChREBP, suppresses cervical cancer cell proliferation, migration and invasion. Our findings provide new ideas for the prevention and treatment of cervical cancer.

Keywords: cervical cancer, ChREBP, MondoA, TXNIP

LncRNA DLGAP1-AS2 modulates glioma development by upregulating YAP1 expression

Wei Miao[1], Ning Li[1], Bin Gu[1], Guoqing Yi[1], Zheng Su[1] and Huilin Cheng[1]

[1]Department of Neurosurgery, Zhongda Hospital, Southeast University, 87 DingJiaQiao Road, Gulou District, Nanjing, Jiangsu 210009, China

LncRNA DLGAP1 antisense RNA 2 (DLGAP1-AS2) is one kind cytoplasmic long non-coding RNA; however, there is rarely little information about its function in physiological process. Here, we demonstrated that LncRNA DLGAP1-AS2 was upregulated in glioma and was quite correlated with poor prognosis of glioma patients. Depletion of DLGAP1-AS2 in glioma cells could inhibit cell proliferation and cell migration, and induce

cell apoptosis, resulting in the suppression of the progression of glioma consequently. Furthermore, knockdown of DLGAP1-AS2 inhibited the growth of xenograft glioma tumour *in vivo* as well. Finally, we verified Yes Associated Protein 1 (YAP1) was the downstream target of DLGAP1-AS2 and DLGAP1-AS2 mo dulated glioma cell proliferation, migration and apoptosis via re gulating YAP1. Our study revealed novel mechanism about how did lncRNA DLGAP1-AS2 execute function in glioma and thus provided potential therapeutic interventions for the treatment of glioma.

Keywords: DLGAP1-AS2, glioma, LncRNA, YAP1

Biochemical and structural analyses of the nucleosome containing human histone H2A.J

Hiroki Tanaka[1,2], Shoko Sato[1], Masako Koyama[1], Tomoya Kujirai[1] and Hitoshi Kurumizaka[1,2]

[1]Laboratory of Chromatin Structure and Function, Institute for Quantitative Biosciences, The University of Tokyo, 1–1–1 Yayoi, Bunkyo-ku, Tokyo 113–0032, Japan, [2]Laboratory of Structural Biology, Graduate School of Advanced Science and Engineering, Waseda University, 2–2 Wakamatsu-cho, Shinjuku-ku, Tokyo 162–8480, Japan

Histone H2A.J, a histone H2A variant conserved in mammals, may function in the expression of genes related to inflammation and cell proliferation. In the present study, we purified the human histone H2A.J variant and found that H2A.J is efficiently incorporated into the nucleosome *in vitro*. H2A.J formed the stable nucleosome, which accommodated the DNA ends. Mutations in the H2A.J-specific residues did not affect the nucleosome stability, although the mutation of the H2A.J Ala40 residue, which is conserved in some members of the canonical H2A class, reduced the nucleosome stability. Consistently, the crystal structure of the H2A.J nucleosome revealed that the H2A.J-specific residues, including the Ala40 residue, did not affect the nucleosome structure. These results provide basic information for understanding the function of the H2A.J nucleosome.

Keywords: chromatin, epigenetics, H2A.J, histone variant, nucleosome

Protein Interaction and Recognition

Molecular basis of fl exible peptide recognition by an antibody

Koki Makabe[1]

[1]Graduate School of Science and Engineering, Yamagata University , 4–3–16 Jyonan, Yonezawa, Yamagata 992–8510, Japan

Antibodies can recognize various types of antigens with high specificity and affinity and peptide is one of their major targets. Understanding an antibody's molecular recognition mechanism for peptide is important for developing clones with a higher specificity and affinity. Here, the author reviews recent progresses in flexible peptide recognition by an antibody using several biophysical techniques, including X-ray crystallography, molecular dynamics simulations and calorimetric measurements. A set of two reports highlight the importance of intramolecular hydrogen bonds that form in an unbound flexible state. Such intramolecular hydrogen bonds restrict the fluctuation of the peptide and reduce the conformational entropy, resulting in the destabilization of the unbound state and increasing the binding affinity by increasing the free energy change. These detailed analyses will aid in the antibody design in the future.

Keywords: hydrogen bond, molecular dynamics, peptide antigen, thermodynamics, X-ray crystal structure

Enzymology

Reaction of threonine synthase with the substrate analogue 2-amino-5-phosphonopentanoate: implications into the proton transfer at the active site

Yasuhiro Machida[1], Takeshi Murakawa[2], Akiko Sakai[1], Mitsuo Shoji[3,4], Yasuteru Shigeta[3] and Hideyuki Hayashi[1]

[1]Department of Chemistry, Takatsuki, Osaka 569–8686, Japan, [2]Department of Biochemistry, Osaka Medical College, Daigamu-machi 2–7, Takatsuki, Osaka 569–8686, Japan, [3]Center for Computational Sciences, University of Tsukuba, Tennodai 1–1–1, Tsukuba, Ibaraki 305–8577, Japan, [4]JST, PRESTO, Honcho 4–1–8, Kawaguchi, Saitama 332–0012, Japan

Threonine synthase catalyses the conversion of O-phospho-L-homoserine and a water molecule to L-threonine and has the most complex catalytic mechanism among the pyridoxal 5′-phosphate-dependent enzymes. In order to study the less-characterized earlier stage of the catalytic reaction, we studied the reaction of threonine synthase with 2-amino-5-phosphonopentanoate, which stops the catalytic reaction at the enamine intermediate. The global kinetic analysis of the triphasic spectral changes showed that, in addition to the theoretically expected pathway, the carbanion is rapidly reprotonated at Cα to form an aldimine distinct from the external aldimine directly formed from the Michaelis complex. The K_d for the binding of inhibit or to the enzyme decreased with increasing pH, showing that the 2-amino-group-unprotonated form of the ligand binds to the enz yme. On the other hand, the rate constants for the proton migrati on steps within the active site are independent of the solvent pH, indicating that protons are shared by the active dissociative gro ups and are not exchanged with the solvent during the course of catalysis. This gives an insight into the role of the phosphate gro up of the substrate, which may increase the basicity of the ε-ami no group of the catalytic lysine residue in the active site.

Keywords: enzyme-inhibitor, proton-migration, pyridoxal-5′-phosphate, threonine synthase, dissociable-group

Biochemistry in Cell Membranes

Characterization of PomA periplasmic loop and sodium ion entering in stator complex of sodium-driven fl agellar motor

Tatsuro Nishikino[1], Hiroto Iwatsuki[1], Taira Mino[1], Seiji Kojima[1] and Michio Homma[1]

[1]Division of Biological Science, Graduate School of Science, Nagoya University, Chikusa-ku, Nagoya 464–8602, Japan

The bacterial flagellar motor is a rotary nanomachine driven by ion flow. The flagellar stator complex, which is composed of two proteins, PomA and PomB, performs energy transduction in marine *Vibrio*. PomA is a four transmembrane (TM) protein and the cytoplasmic region between TM2 and TM3 ($loop_{2–3}$) interacts with the rotor protein FliG to generate torque. The periplasmic regions between TM1 and TM2 ($loop_{1–2}$) and TM3 and TM4 ($loop_{3–4}$) are candidates to be at the entrance to the transmembrane ion channel of the stator. In this study, we purified the stator complex with cysteine replacements in the periplasmic loops and assessed the reactivity of the protein with biotin maleimide (BM). BM easily modified Cys residues in $loop_{3–4}$ but hardly lab elled Cys residues in $loop_{1–2}$. We could not purify the plug deletion stator (ΔL stator) composed of $PomB_{Δ41–120}$ and WT-PomA but could do the ΔL stator with PomA-D31C of $loop_{1–2}$ or with PomB-D24N of TM. When the ion channel is closed, PomA and PomB interact strongly. When the ion channel opens, PomA interacts less tightly with PomB. The plug and $loop_{1–2}$ region regul ate this activation of the stator, which depends on the binding of sodium ion to the D24 residue of PomB.

Keywords: flagellar stator, periplasmic loop, plug region, PomAB, Vibrio

CELL

Cell Death

miR-206 regulates non-small-cell lung cancer cell aerobic glycolysis by targeting hexokinase 2

Ke-Gang Jia[1], Gang Fen[1]g, Yu-Suo Tong[2], Guang-Zhou Tao[2] and Lian Xu[3]

[1]Department of Thoracic Surgery, Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital, 32 First Ring Road, Qingyang district, Sichuan 610072, China, [2]Department of Radiation Oncology, The Affiliated Huaian No. 1 People's Hospital of Nanjing Medical University, 1 Huanghe West Road, Huaiyin district, Huaian 223300, China, [3]Department of Rehabilitation, Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital, 32 First Ring Road, Qingyang district, Sichuan 610072, China

Aerobic glycolysis was closely associated with the malignant transformation and prognosis of tumours. miR-206 was found to be downregulated in several cancers. However, whether miR-206 functions in non-small-cell lung cancers (NSCLCs) via the process of aerobic glycolysis remains poorly characterized. Quantitative real-time PCR was performed to detect miR-206 level in NSCLC cells and tissues. The effect of miR-206 on hexokinase 2 (HK2) expression was examined through miR-206 overexpression or miR-206 knockdown. CCK-8 assay and colony formation assay were carried out to explore the role of miR-206 on cell proliferation and colony formation, respectively. The relationship between miR-206 and HK2 was measured by dual-luciferase reporter assay. Glucose consumption, lactate production assay and ATP generation were performed in NSCLC cells following miR-206 and HK2 overexpression. We found that miR-206 was downregulated in NSCLC tissues and cells. miR-206 overexpression downregulated the expression of HK2 via targeting HK2 3′UTR in NSCLC cells. In addition, miR-206 decreased the cell viability and colony formation in NSCLC cells. Furthermore, miR-206 reduced glucose uptake, lactate production and ATP generation in NSCLC cells via HK2 repression. In conclusion, these findings suggested that miR-206 regulated NSCLC cell aerobic glycolysis by targeting HK2.

Keywords: glycolysis, hexokinase 2, miR-206, non-small-cell lung cancer, proliferation

lncRNA CCAT1/miR-490-3p/MAPK1/c-Myc positive feedback loop drives progression of acute myeloid leukaemia

Chenghong Wang[1], Fangping Chen[1], Zili Fan[1], Chenjiao Yao[1] and Lijun Xiao[1]

[1]Department of Hematology, The 3rd Xiangya Hospital, Central South University, No. 138, Tongzipo Road, Yuelu District, Changsha, Hunan 410013, China

Acute myeloid leukaemia (AML) is a frequently diagnosed malignancy in adults. Long non-coding RNA (lncRNA) colon cancer-associated transcript 1 (CCAT1) has been well known to play vital roles in multiple malignancies including AML. Unfortunately, the detailed mechanism of CCAT1 in AML progression remains obscure. In this study, we demonstrated that CCAT1 was up-regulated in AML samples while its target, miR-490-3p, was relatively down-regulated. CCAT1 markedly increased viability and metastasis of AML cells, while miR-490-3p had opposite effects. CCAT1 could specifically bind to miR-490-3p and reduce its expression and activity, and MAPK1 was a target gene of miR-490-3p. Overexpressed CCAT1 could induce MAPK1 expression and c-Myc reciprocally increased CCAT1 expression. Our data implied that miR-490-3p could be a novel therapeutic target for AML, and highlights the crucial role of CCAT1/miR-490-3p/MAPK1/c-Myc positive feedback loop in AML progression.

Keywords: AML, c-Myc, CCAT1, MAPK1, miR-490-3p

BIOTECHNOLOGY

Biotechnology General

A monoclonal antibody recognizing a new epitope on CD81 inhibits T-cell migration without inducing cytokine production

Takuya Hasezaki[1], Tadahiko Yoshima[2], Mikael Mattsson[3], Anna Särnefält[3] and Keiko Takubo[4]

[1]External Innovation, Sumitomo Dainippon Pharma Co., Ltd, 3–1–98 Kasugade Naka, Konohana-ku, Osaka 554–0022, Japan, [2]Applied Bioscience Group, Bioscience Research Laboratory, Sumitomo Chemical Co., Ltd., 3–1–98 Kasugade Naka, Konohana-ku, Osaka 554–0022, Japan, [3]BioInvent International AB, Sölvegatan 41, SE-223 70 Lund, Sweden, [4]Group II, Platform Technology Research Unit, Sumitomo Dainippon Pharma Co., Ltd., 3–1–98 Kasugade Naka, Konohana-ku, Osaka 554–0022, Japan

CD81 is involved in leukocyte migration and cytokine induction. Previous work found that anti-CD81 monoclonal antibodies (mAbs) showed therapeutic potential for several immune diseases via inhibiting leukocyte migration. Although the suppression of cell migration is a promising approach for treating immune diseases, some anti-CD81 mAbs can induce cytokine production, which may exacerbate disease. To obtain new anti-human CD81 mAbs that inhibited migration in the absence of cytokine production enhancement activity, we screened a human single chain variable fragment by phage library. One of the new anti-CD81 mAbs isolated, DSP-8250, had equivalent inhibitory cell migration activity with the established anti-CD81 mAb 5A6, but it lacked cytokine induction activity. These mAbs recognized different epitopes on CD81. mAb 5A6, which had inhibitory activity on T-cell migration and increased cytokine production, bound to three residues, Ser179, Asn180 and Phe186 of CD81. In contrast, DSP-8250, which had inhibitory activity on T-cell migration but no cytokine enhancement activity, bound to four residues, His151, Ala164, Ser168 and Asn172 of CD81 as a unique epitope. These results indicate that the set of His151, Ala164, Ser168 and Asn172 forms a novel epitope that might make the application of anti-CD81 mAb therapeutically useful.

Keywords: CD81, epitope, monoclonal antibody, phage display, site-directed mutagenesis